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SUBTITLE: Percutaneous Absorption of Carbon-14 Labeled Trinitrotoluene From Air and Soil

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13. ABSTRACT *(Maximum 200 words)* The percutaneous absorption of carbon-14 labeled trinitrotoluene (TNT) from various media (air, water, acetone and soil) and carbon-14 TNT or its degradation products from compost was determined using excised skin mounted in skin penetration-evaporation chambers. Appearance of label in tissue culture media was used to measure the steady state absorption rate of TNT from saturated air or water. Radiolabel recovered from the dermis and tissue culture media was used to determine percent absorption from acetone, soil and compost.

Due to low vapor concentration, the absorption rate of TNT from saturated air was found to be insignificant. The permeability coefficient of TNT from saturated water was used to derive a theoretical absorption of 98% from soil, which overestimated experimental values (3-4%). Absorption from acetone was 25% of applied dose. Percent absorption of radiolabel from compost (0.2-0.3%) was significantly lower than from soil. Skin absorption measurements in the live pig for soil (4%) and compost (1%) confirmed in vitro findings. In summary, the study demonstrated the value of the in vitro model to predict in vivo results. As measured by percutaneous absorption of radioactivity, the composting process significantly reduced the bioavailability of TNT or its degradation products in soil.

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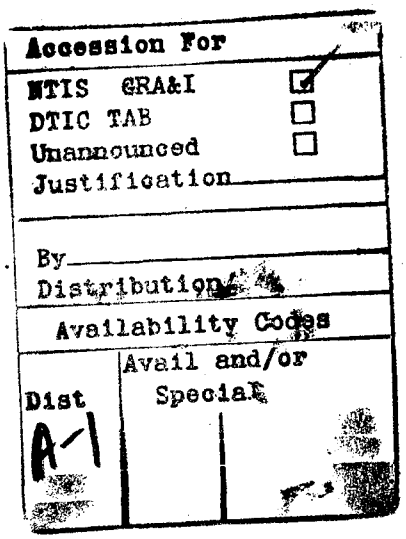

William C. Reynolds April 13, 1994
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INTRODUCTION

Bioremediation of soils is becoming increasingly important for biodegradation of pollutants, including many of our most toxic and environmentally persistent pollutants[1]. Composting is being considered as a method for bioremediating trinitrotoluene (TNT)-contaminated soils on military bases. During the composting process, TNT becomes "bound" to organic materials in the compost and cannot be extracted with aqueous solutions or organic solvents[2]. To evaluate risks associated with skin contact with composts of TNT-contaminated soil, we assessed the skin absorption of radiolabeled TNT from air, soil and compost.

Skin contact with contaminated soil can represent a significant route of exposure to hazardous compounds[3]. Studies have been conducted with soils contaminated with DDT, TCDD, heavy metals and aromatic solvents. Results to date indicate that soil does not enhance contaminant absorption through the skin; however, under certain experimental conditions, levels of chemical absorption from soil may approach the values obtained after topical exposure to the pure compound.

Skin absorption of chemical vapors requires that the chemical be capable of penetrating the skin and of reaching a sufficient concentration on the skin surface to achieve a significant driving force for skin penetration. Due to low volatility, some chemicals simply cannot achieve vapor concentration sufficient to pose a hazard by dermal absorption. Vapor absorption through skin is many times ignored in risk assessments because it is generally assumed

that pulmonary uptake is much larger than dermal absorption. For example, Blank and McAuliffe[4] estimated that an adult with 2 square meters of skin surface area in air containing 10 ppm benzene would absorb 7.5 uL of benzene from inhalation and 1.5 uL from body exposure after an exposure lasting 1 hour. However, with certain occupational or environmental exposures, skin vapor absorption could be the primary route of exposure. An example would be individuals having only respiratory protection and entering environments with high air concentrations of toxics. Riihimaki and Pfaffli[5] reported that subjects exposed to 20 ppm xylene only by inhalation absorbed, on average, 357 umole of the compound over a 3.5 hour period. Over a similar period, subjects using respiratory protection absorbed, via the skin, 197 or 419 umoles of xylene when exposed to 300 or 600 ppm xylene. These results show that a worker could absorbed xylene through the skin in amounts comparable to those achieved via the respiratory tract at lower concentrations.

There is controversy concerning the appropriate methodology for experimental determinations of percutaneous absorption of chemicals in soil. The "permeability coefficient" approach involves the determination of a flux value, normalized for concentration, that represents the rate at which the chemical penetrates the skin (cm/hour). The "percent absorbed" approach involves the determination of the fraction of the applied dose absorbed across the skin in a specified time. To be meaningful, the duration of exposure, the amount of material applied per unit area, and the area exposed need to be specified. The permeability

coefficient approach is advocated over the percent absorbed approach for determining the dermally absorbed dose of compounds in an aqueous media or air. Because of the lack of data demonstrating the scientific reliability of using aqueous permeability coefficient data for compounds bound to soil and reduced uncertainty in defining an applied dose, the percent absorbed approach is presently recommended by the EPA for determining the dermally absorbed dose of soil contaminants[6].

Therefore, the percent skin absorption of ^{14}C-TNT from soil was measured by both in vitro and in vivo methods. Similar determinations were made from compost to assess the bioavailability of ^{14}C-TNT or its degradation products present in the bioremediated soil. In vitro percent absorption measurement of TNT from acetone was also done to provide an upper bound for comparison with soil and compost.

The permeability coefficients (K_p) of TNT absorption from water and air were also determined. The K_p^{water} was used to predict a percent absorption from a theoretical model[6] for comparison to measured values. The K_p^{air}, together with the saturated vapor concentration of TNT in air, were used to assess the importance of skin absorption of TNT from vapor exposure.

Because of the importance of making experimental determinations of dermal exposure which reflect the conditions of actual exposure and do not over- or under-estimate exposure due to experimental artifact, we employed an in vitro skin penetration-evaporation model (Figure 1) utilizing pig skin. This model has

been shown to be predictive of human skin absorption[7].

METHODS

Chemicals and Test Samples of Soil and Compost: [RING-^{14}C(U)]2,4,6-trinitrotoluene (TNT), specific activity of 17.9 mCi/mmole in tetrahydrofuran solution (25.4 mg/ml), was obtained from Dupont-New England Nuclear, Boston, MA with reported radiochemical purity of 97.8%. Uncontaminated soil from Umatilla Army Depot (UMDA) was supplied by US Army Biomedical Research and Development Laboratory, Fort Detrick, Frederick, MD. Prior to each experiment, soil was mixed with ^{14}C-TNT solution and the tetrahydrofuran was allowed to evaporate, giving a final activity of 1.10-1.52 mCi per gram soil. Uncontaminated soil from UMDA spiked with ^{14}C-TNT and composted to give a final activity of 1.6 mCi per gram of compost was supplied by the US Army Biomedical Research and Development Laboratory.

Animals: Specific pathogen-free female Yorkshire pigs, weighing 15 to 20 kg, were used in the study.

In Vitro Percent Absorption Measurements: The basic procedure consisted of obtaining freshly excised skin, removing a portion of the dermis and subcutaneous fat with a dermatome, and mounting the skin on a penetration cell (Figure 1) so that the visceral side of the skin was in contact with tissue culture medium perfusing the penetration cell. Using a fraction collector, medium exiting the penetration cell was fractionated into hourly or bihourly samples, which were analyzed for radiolabeled penetrant. An evaporation cell or donor cell (Figure 1) was mounted on the outer surface of

the skin. The skin area exposed by the evaporation cell (0.8 cm^2) was used for chemical application. The evaporation cell maintained a 600 ml/min air flow over the skin surface and contained replaceable traps for collection of chemical which evaporated from the skin surface. Details of the procedure have been published[8], and were used with the following modifications. Pig skin was dermatomed to a thickness of 0.5 mm. The application area was pretreated with 5 ul of artificial sweat[9] prior to application of soil or compost. Twelve hours after application, soil or compost particles were removed from the skin surface by wiping twice with a dry cotton ball. Cotton balls were placed in liquid scintillation counting fluid (Ultima Gold, Packard Instruments, Downers Grove, IL) for radiometric assay. Forty eight hours after application, skin was removed from the diffusion cells and the epidermis was covered with a polyvinyl film (Stretch-tite plastic food wrap for microwave ovens, Sutton, MA). A brass weight heated to 65°C was pressed against the epidermis for 90 seconds. The epidermis was then teased from the dermis with a forceps. Epidermis and dermis were placed in separate liquid scintillation counting (LSC) vials and solubilized with approximately 1 ml of hyamine solution (tissue solubilizer, Packard Instruments) heated to 50-60°C for approximately 1 hour. Ten ml of LSC counting fluid was added to each vial for radiometric assay using a Packard Model CA1900 scintillation counter.

Six evaporation/penetration cells (A-F) were assembled on each day that a pig skin sample was obtained. Cell A received low-dose

soil (1.7 ± 0.3 mg/cm^2), cell B received high-dose soil (5.3 ± 0.8 mg/cm^2), cell C received low-dose compost (1.3 ± 0.3 mg/cm^2), cell D received high-dose compost (5.4 ± 0.6 mg/cm^2), and cells E and F received acetone solution (10 ug/cm^2 ^{14}C-TNT in 6.25 ul acetone/cm^2). This procedure was repeated on five separate days with skin from a different pig to give a maximum of 6 replicates for each soil or compost exposure and 12 replicates for the acetone solution exposure.

In Vitro K_p Measurements: Three evaporation/penetration cells (G,H and I) were assembled on the day that a pig skin sample was obtained. Cell G was used to expose the skin to air saturated with ^{14}C-TNT vapor at 20°C and was modified as follows. The air inlet ports of the evaporation chamber (Figure 1) were sealed and a well was created at the tip of the vapor trap for placement of ^{14}C-TNT solution. A cotton plug was inserted into the top of the vapor trap approximately 2 cm from the well and the vapor trap was filled to the top with 1/8" silica gel beads (Type II, Sigma, St. Louis). The silica gel prevented accumulation of moisture in the evaporation chamber. Five ul of ^{14}C-TNT in tetrahydrofuran (127 ug of TNT) was placed in the vapor trap well with a syringe (Hamilton, Reno, NV). This amount of TNT insured no donor cell depletion and allowed the generation of a steady state absorption rate during the 48 hour test period. Tetrahydrofuran was allowed to evaporate and the vapor trap was placed in the evaporation chamber. Water at 20°C circulated through the jacket of the evaporation cell.

Cells H and I were used to expose the skin to a saturated aqueous solution of ^{14}C-TNT and were modified as follows. The evaporation chamber was replaced with a donor chamber (Laboratory Glass Apparatus, Berkeley, CA) fitted with a 14/20 stopper . Five hundred ul of water saturated with ^{14}C-TNT was placed in the donor chamber with a Hamilton syringe. The solution was replaced with an equal volume of fresh saturated solution at 12 hours to prevent donor cell depletion and to achieve a steady state absorption rate in the 24 hour test period.

To calculate the absorption rate of TNT through pig skin, the output of penetrant from the cells was added to the penetrant remaining in the receptor chamber at the various time points. The latter quantity was estimated by assuming that the concentration of penetrant in any given output sample represented the concentration of penetrant at the midpoint of the time interval over which the output sample was collected. The concentration in the receptor compartment at the end of the sampling period can be derived by interpolation between successive output sample concentrations.

Linear regression equations derived from the steady state portion of individual cumulative absorption curves were used to calculate mean steady state absorption rates of TNT from air and water. These values were divided by the respective saturation concentrations to obtain values for K_p^{air} and K_p^{water}.

Soil/Water Partition Coefficient: The procedure of Pennington and Patrick[10] was followed. Uncontaminated soil (approximately 50 mg) was added to a 2 ml vial containing 1.0 ml of an aqueous

solution of 20 ug/ml ^{14}C-TNT. The vial was placed inside a water jacket at 20°C and the slurry was mixed for 8 hours with a magnetic stirrer. The mixture was filtered and an aliquot of the filtrate counted by LSC. The uptake of TNT by soil was determined from the decreased TNT concentration in the water phase. The partition coefficient, 3.72 $\pm$ 0.94 L/Kg, was calculated from 4 replicates by dividing the soil concentration (mg/Kg) by the water concentration (mg/L).

In Vivo Percent Absorption Measurements: In vitro data were confirmed by determining percutaneous penetration of radiolabel from the 1.7 mg/cm^2 soil dose and the 1.2 mg/cm^2 compost dose in the live pig (4 replicates each). A clearance study involving subcutaneous administration of the pure compound (76 ug TNT in 1.0 ml propylene glycol) in four additional animals was necessary to generate the "percent absorbed" data for TNT in soil. Published procedures[11] were used with the following modifications. The application area was pretreated with 125 ul of artificial sweat prior to application of soil or compost. Twelve hours after application, soil or compost particles were removed from the skin surface by wiping twice with a dry cotton ball. Cotton balls were placed in liquid scintillation counting fluid (Ultima Gold, Packard Instruments, Downers Grove, IL) for radiometric assay. Protective patches were replaced at 12 hours and removed at 48 hours. Feces were homogenized with an approximately equal volume of water and a 5.0 gram aliquot was mixed with 5 grams of methanol and the mixture allowed to stand overnight. Liquid was vacuum filtered from

particulate with Whatman No. 1 filter paper. A 1 gram aliquot of the filtrate was added to 10 ml of LSC fluid for radiometric assay. Triplicate samples of various tissues weighing 0.5 to 1.0 gram were dissolved in approximately 1 ml of hyamine solution (Tissue solubilizer, Packard) and the samples decolorized with up to 1 ml of 30% hydrogen peroxide solution (Fisher). Samples were allowed to stand at room temperature for at least 24 hours to allow for decay of chemiluminescence. Samples were counted with a protocol to correct for chemiluminescence and DPM determined using an extended range of quench standards. Application site skin and fat residues were calculated from DPM/gram values times the application site area (25 cm^2) and layer thickness (0.2 cm for skin and 1.0 cm for fat) and assuming a tissue density of 1. Values for liver, kidney, spleen, skeletal muscle and subcutaneous fat were calculated from DPM/gram values times the wet tissue weights. Weights for skeletal muscle and subcutaneous fat were determined from the percent body composition of pigs 28 days postnatal[12] times the total weight of each animal. Percutaneous absorption for TNT in soil was calculated by dividing the sum of the radioactivity recovered in the urine and feces by the amount of applied radioactivity and dividing the quotient by fraction of radioactivity (fraction of injected dose) recovered in the urine and feces after subcutaneous TNT injection. Since the bioremediation process likely generated degradation products of TNT in the compost, percutaneous absorption was calculated as percentage of radioactivity recovered in urine, feces, application

site fat, liver, kidney, spleen, skeletal muscle and fat.

Calculation of Theoretical Percent Absorption[6]:

$$ABS = \frac{(\text{soil density})\ (K^{soil}p,s)}{(AF)\ (k_{soil} + k_{vol})}\ [1-e^{(ksoil + kvol)tevent}]$$

$$= 0.98$$

where

ABS = Absorbed fraction

soil density = bulk soil density, assumed to be 1.35 g/cm^3

$K^{soil}_{p,s}$ = skin permeability coefficient for TNT in soil
= 3.06 x 10^{-3} cm/hour, calculated from the equation:

$$K^{soil}_{p,s} = \frac{K^{water}p,s}{K_{soil/water}}$$

where $K^{water}_{p,s}$ is the skin permeability coefficient for TNT in water, measured to be 11.4 x 10^{-3} cm/hour and

$K_{soil/water}$ is the soil/water partition coefficient for TNT, measured to be 3.72 (unitless)

k_{soil} = $(K^{soil}_{p,s})$ (soil density) (1000) / (AF) = 2.43 hour^{-1}

where AF equals the soil adherence factor, set to the value of 1.7 mg/cm^2, the soil dose in vitro and in vivo

k_{vol} = $(K_h)\ (D_{air})$ (3600 sec/hour) / (AF) (K_D) (1) = 0.0541 hour^{-1}

where K_h = dimensionless Henry's law constant, calculated

from the equation $K_h = H/RT$

where H is Henry's law constant, calculated from

H = vapor pressure in atm/water solubility in mole/m^3

R= 8.205 x 10^{-5} m^3 atm/ mole degree K

T= 293 deg K (20°C)

The vapor pressure was calculated to be 1.06 x 10^{-5} atm by the Modified Watson Correlation[13], and the water solubility measured at 20°C at saturation as 1.34 mg/100 ml or 0.590 mole/m^3

D_{air} = air diffusivity = 6.34 x 10^{-2} cm^2/sec, estimated by the method of Fuller, Schettler and Giddings[13]

K_D = soil/water partition coefficient, 3.72 L/Kg

l = thickness of boundary layer at air-soil interface and assigned a value of 0.5 cm, the default value used by McKone[6].

t_{event} = length of exposure event, which was 12 hours (in vitro and in vivo)

Statistical Analysis: For in vitro determinations of percent evaporation and percent recovery of radioactivity in the receptor fluid or dermis following skin application of radiolabeled compost and soil at 2 doses (Table 1), data were compared using two-way analysis of variance (ANOVA), assuming non-equality of variances. When a significant F occurred, Tukey's test was employed to determine which means were significantly different. Percent evaporation and penetration (into receptor fluid or dermis) of ^{14}C-TNT from acetone solution were compared to the corresponding soil and compost data (Table 1) using one-way ANOVA. When a significant F occurred, Dunnett's test was used to determine which values were significantly different from the acetone data.

Percutaneous penetration of soil and compost, each determined in vitro and in vivo (Table 5), were compared using two-way ANOVA and Tukey's test. K_p^{air} and K_p^{water} (Table 2) were compared using the Student t test. All determinations were carried out at the 0.05 level of significance.

RESULTS AND DISCUSSION

Evaporation and penetration into the dermis and receptor fluid are the major factors causing the loss of TNT from the skin

surface, as shown by the in vitro distribution of radiolabel following topical application of acetone solution (Table 1). When the carrier was soil rather than acetone, loss by these routes decreased and more radiolabel was recovered by skin decontamination. Following composting of soil, loss of radiolabel by evaporation or penetration was almost insignificant, with skin decontamination accounting for the bulk of label recovery. The composting process may change the chemical structure of the radiolabel, making it less volatile, less permeable through skin, or more tightly bound to the soil particles.

Increasing the dose of soil resulted in a significant decrease in mean percent loss of TNT by evaporation, while penetration remained nearly the same. The higher dose of compost did not have a significant effect on loss of radiolabel by evaporation or penetration.

Previous studies have shown that in vivo percutaneous penetration can best be predicted from in vitro data by adding penetrant residues found in the dermis to the amount of penetrant found in receptor fluid[7]. In this study, predicted in vivo absorption would be 0.2-0.4 percent for TNT or degradation products in compost, 3-4 percent for TNT in soil, and 25 percent for TNT in acetone solution.

Skin penetration constants for in vitro absorption of TNT through pig skin are given in Table 2. Although the K_p values for TNT from air was lower than from water ($p < 0.05$), the much lower saturation concentration in air versus water was primarily

responsible for the much lower absorption rate from air.

The aqueous K_p was used to calculate a theoretical percent absorption of TNT from soil of 98 percent (see Methods), which was a significant overestimation of the observed value for soil of 3-4 percent based on in vitro data (Table 1).

The distributions of radioactivity 96 hours after topical application of ^{14}C-TNT in compost and soil and subcutaneous injection of ^{14}C-TNT in propylene glycol are given in Tables 3 and 4. The major route of excreted radioactivity was urine in each case. Radioactivity recovered from the protective patch accounted for the majority of label recovered from the topical exposures. More radiolabel was present in skeletal muscle and fat from compost-treated pigs than from soil-treated pigs, despite the fact that absorption of radioactivity was significantly lower for compost treatment vs soil treatment. This change in tissue affinity suggests that the composting process changed the chemical identity of the label.

In the pig, there did not appear to be any direct toxic effect of the soil or compost on the skin itself, as observations of soil and compost treated skin sites at 12 hours and 48 hours were unremarkable.

In vitro vs in vivo absorption values for the low doses of soil and compost were not significantly different (Table 5), with the in vivo results confirming the differences between soil and compost observed in vitro.

CONCLUSIONS

The results of this study demonstrated the value of the in vitro model to predict in vivo results. As measured by the percutaneous absorption of radioactivity, the composting process significantly reduced the bioavailability of TNT or degradation products in soil. Absorption of TNT vapor by the skin was limited by the low vapor concentration that TNT could achieve in air. Finally, a theoretical model used to predict skin absorption of TNT from soil led to a large overestimate of experimentally determined values.

Table 1. Disposition of C-14 labeled Trinitrotoluene Following Topical Application in Acetone Solution, Soil and Compost to Excised Pig Skin

Test Material	Carrier Dose (mg/cm^2)	TNT Eq. (ug/cm^2)	Percent of Applied Radioactive Dose ± 1 S.D.						
			Evap.[a]	Receptor Fluid[a]	Skin Decon.	Epi.	Derm.[a]	Total Recovery[b]	N
Acetone solution	6.25 ul/cm^2	10±2	44±9	24±6	5±2	7±2	1.4±0.5	82±9	11
Soil	1.7±0.3	36±7	15±5	3±2^x	29±8	1.0±0.7	0.6±0.5^x	45±9	5
Soil	5.3±0.8	117±25	5±3	2.2±0.8^x	37±16	2±3	0.6±0.3^x	48±17	6
Compost	1.3±0.3	10±2	0.02±0.02^x	0.03±0.02^y	18±9	2±2	0.2±0.2^x	19±10	6
Compost	5.4±0.6	43±4	0.05±0.05^x	0.01±0.00^y	35±9	2±2	0.3±0.2^x	38±9	6

[a]Within a given column, values with the same superscript (e.g. x) are not significantly different ($p > 0.05$).

[b]Low total recovery for soil and compost test materials was due to loss of soil or compost particles during skin decontamination with a dry cotton ball and due to incomplete partitioning of TNT from soil or compost particles into liquid scintillation counting solution.

Table 2. Saturation Concentration, Steady State Absorption Rate, Lag Time, and Permeability Coefficient for In Vitro Absorption of TNT through Pig Skin[a]

Medium	Saturation Concentration	Steady State Absorption rate	Lag Time	K_p	N
Air	0.493 ug/cm^3	2.0 $\pm$ 0.9 ng/cm^2-h	10 $\pm$ 3 h	4 $\pm$ 2 x 10^{-3} cm/h	4
Water	134 ug/ml	1.5 $\pm$ 0.7 ug/cm^2-h	0.9 $\pm$ 0.7 h	11 $\pm$ 5 x 10^{-3} cm/h	10

[a]Where indicated, values are mean $\pm$ 1 S.D.

Table 3. Disposition of Radioactivity in Pigs 96 Hours after Subcutaneous Administration of C-14 Labeled Trinitrotoluene (TNT) and Topical Application in Soil and Compost Samples.

Test Material	Compost or Soil Dose (mg/cm^2)	Percent of Applied Radioactive Dose ± 1 S.D.							
		Urine	Feces	Skin Decon	Skin Patch	Application Site Skin	Application Site SubQ. Fat	Total Recovery[a]	N
^{14}C-TNT in Compost	1.15±0.07	0.13±0.06	0.00±0.00	4±2	4±1	1.2±0.9	0.17±0.06	10.6±0.7	3
^{14}C-TNT in Soil	1.66±0.09	2.2±0.2	0.06±0.04	2±1	40±22	2±1	0.15±0.08	46±21	4
^{14}C-TNT Subcut.	-	68±10	2+2	-	-	-	-	71±9	4

[a]Low total recovery for soil and compost test materials was due to loss of soil or compost particles during skin decontamination with a dry cotton ball and due to incomplete extraction of radioactivity from soil or compost particles. Total recovery includes small percentages recovered from various tissues (see Table 4).

Table 4. Radioactive Residues in Various Tissues of the Pig 96 Hours after Subcutaneous Administration of C-14 Labeled Trinitrotoluene (TNT) and Topical Application in Soil and Compost Samples.

Test Material	Percent of Applied Radioactive Dose[a]				
	Liver	Kidney	Spleen	Skeletal Muscle	Subcut. Fat
^{14}C-TNT in Compost	0.00±0.01	0.00±0.00	0.00±0.00	0.4±0.2	0.6±0.4
^{14}C-TNT in Soil	0.01±0.02	0.00±0.00	0.00±0.00	0.01±0.01	0.13±0.06
^{14}C-TNT Subcut.	0.2±0.1	0.01±0.02	0.00±0.00	0.3±0.2	0.8±0.2

[a]Mean ± S.D. of 4 replicates of the mean of triplet determinations for each tissue.

Table 5. In Vitro and In Vivo Percutaneous Absorption of C-14 TNT Residues in the Pig from Soil and Compost.

Test Material	Carrier Dose (mg/cm^2)	TNT Eq. (ug/cm^2)	Percutaneous Absorption[a]	N
Compost In Vitro	1.3±0.3	10±2	0.2 ± 0.2^{x}	6
Compost In Vivo	1.2±0.1	9.3±0.6	1.2 ± 0.1^{x}	3
Soil In Vitro	1.7±0.3	36±7	4 ± 2^{y}	5
Soil In Vivo	1.7±0.1	27±2	3 ± 2^{y}	4

[a]Percent of applied radioactive dose. Percutaneous absorption in vitro is the sum of radioactivity recovered from the receptor fluid and the dermis. Percutaneous absorption of ^{14}C-TNT in soil in vivo is the sum of radioactivity recovered from the urine and feces divided by the corresponding sum recovered after subcutaneous administration of ^{14}C-TNT and multiplication of the quotient by 100. Percutaneous absorption of radioactivity from compost in vivo is the percent recovery of radioactivity from urine, feces, application site fat, subcutaneous fat, liver, kidney, spleen and skeletal muscle. Values within the column having the same superscript were not significantly different.

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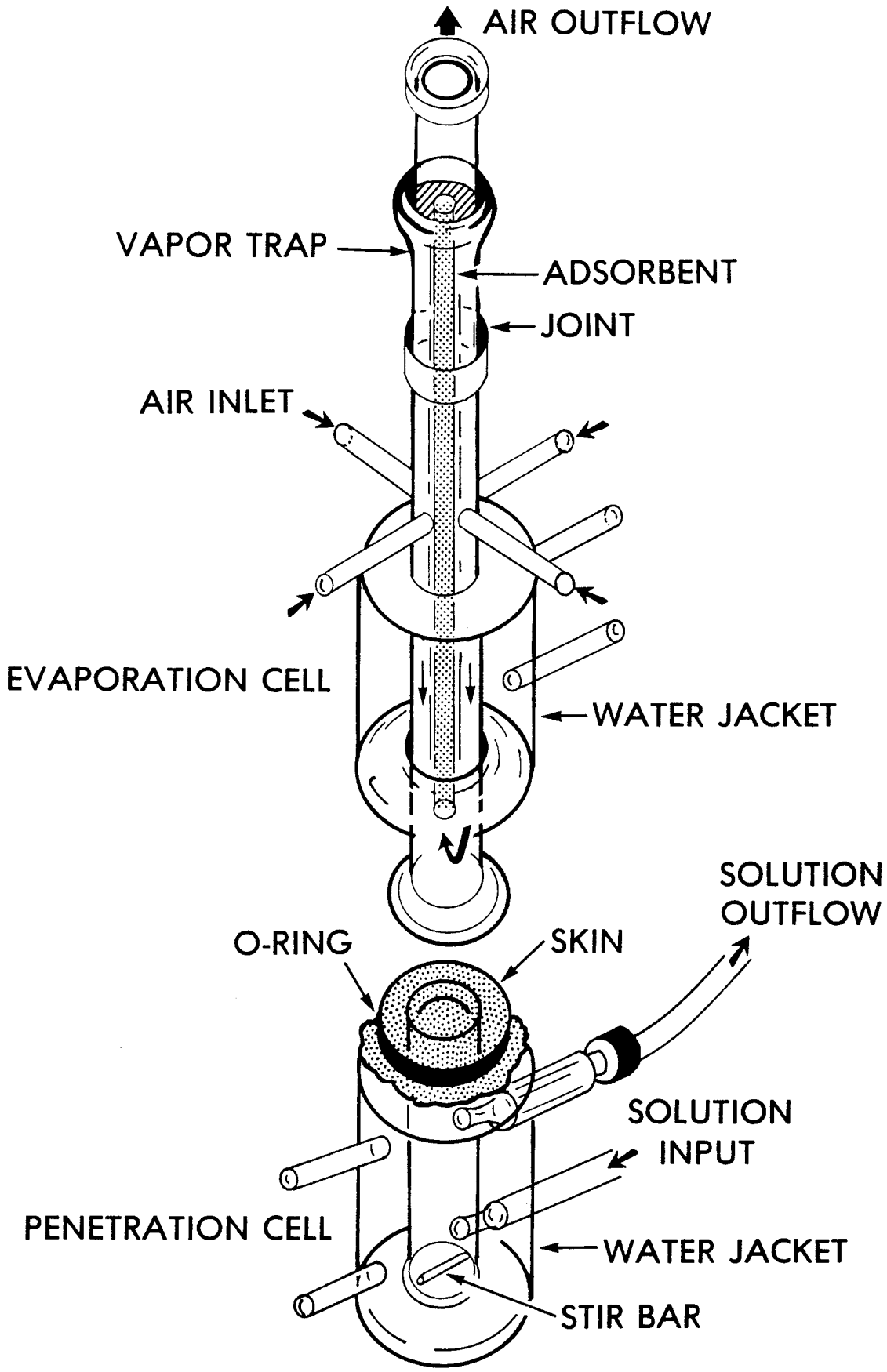

Figure 1. Skin Penetration-Evaporation Cells